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Dravet syndrome: Patients with co-morbid *SCN1A* gene mutations and mitochondrial electron transport chain defects

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ABSTRACT

Purpose: To review our cohort of patients with Dravet syndrome and determine if patients with *SCN1A* mutations can also express mitochondrial disease due to electron transport chain dysfunction.
Methods: A retrospective chart review was used to describe clinical manifestations and retrieve biochemical testing, neuroimaging, gene sequencing, and electroencephalographic results of patients expressing both mitochondrial disease and Dravet syndrome.
Results: Two children were found to have pathological mutations in the *SCN1A* gene and defects in mitochondrial electron transport chain complex activity. Both developed early febrile and medically intractable afebrile seizures with resulting neurocognitive decline. In the first patient, a muscle biopsy demonstrated complex IV dysfunction and in the second patient, complex III dysfunction. Patient 1 had more difficult to control seizures, and had features consistent with severe autism. Patient 2, who had earlier control and less severe seizures, did not have features of autism. Patient 1 had *SCN1A* missense mutation, c. 3734 G > A and patient 2 had a mutation, c. 3733 C > T, which produces a truncation mutation.
Conclusion: Our two patients underscore the need to rule out possible co-morbid mitochondrial disease and Dravet syndrome. The treatment of seizures for each is different, with valproic acid being first line treatment in Dravet syndrome and contraindicated in many mitochondrial diseases, due to possible induction of liver failure and death. Failure to pursue complete diagnostic evaluation might influence medication choice, possible seizure control, and developmental outcomes.

1. Introduction

Dravet syndrome, a catastrophic epilepsy syndrome, is associated with mutations in the *SCN1A* gene in up to 90% of cases.[1,2] Development in the first year of life is typically normal, but early febrile and subsequent afebrile intractable seizures lead to developmental regression, ataxia, and autistic-like behaviors.[3,4] Mitochondrial disease can present similarly.[5–8] Recently, a single patient was reported to have mutations in the mitochondrial DNA replicase and repair enzyme, polymerase gamma 1 (*POLG*) and pathological mutation in *SCN1A*, but with normal mitochondrial electron transport chain activities.[9] This case demonstrated that both mitochondrial disease and Dravet syndrome may co-exist. We present two children with co-morbid *SCN1A* mutations and definite mitochondrial disease[10] due to electron transport chain deficiencies, and highlight the similar clinical manifestations, including autistic features of both these diseases.

2. Methods

We reviewed our cohort of 24 patients with pathological mutations in the *SCN1A* gene for possible mitochondrial disease. This study was approved by our Institutional Review Board. Childhood Autism Rating Scale [CARS[11]] assessment was performed using clinical observation, chart review and parental interview. The CARS consists of 14 domains assessing behaviors associated with autism. Total scores can range from a low of 15 to a high of 60; scores below 30 indicate that an individual is in the non-autistic range, scores between 30 and 36.5 indicate mild to moderate autism and scores from 37 to 60 indicate severe autism.[12] CARS testing was carried out by one of the authors (A.K.C.). *SCN1A* sequencing was performed commercially (Athena Diagnostics, MA). Electron transport chain enzyme activities were obtained from flash frozen, −80°, vastus lateralis muscle biopsy

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specimens and tested commercially (Center for Inherited Disorders of Energy Metabolism (CIDEM), Cleveland, OH).

3. Results

3.1. Patient 1

Patient 1 is now 15-years old. He initially presented with 2 febrile seizures and subsequent afebrile seizures around 9 months of age. His seizures became intractable and he was admitted 9 times for tonic clonic status epilepticus, with the last status epilepticus event occurring around age 4 years. His status epilepticus events had duration of up to 2.5 h. He required insertion of a gastrointestinal-tube for nutrition due to failure to gain weight. He developed an intention tremor at age 3 years and has had severe constipation his whole life. His seizures have evolved into staring "spells" and drop seizures. Serial electroencephalograms (EEG) were notable for background slowing and bilateral independent temporal onset of seizures captured by Video-EEG monitoring. Brain magnetic resonance imaging (MRI) scans performed at age 2, 4, and 8 years were interpreted as without abnormality. Until seizure onset, early development was normal. He was initially treated with phenobarbital, and subsequently with carbamazepine, lamotrigine, clonazepam, topiramate and zonisamide without seizure control. A vagus nerve stimulator was implanted at age 7-1/2 years without change in seizure frequency. Currently, he is having 1 short, complex partial seizure every two weeks while on the combination of valproic acid, zonisamide, and lamotrigine. He was started on valproic acid before muscle biopsy results returned several years ago.

The family history was significant for epilepsy. The patient's father had a seizure disorder since 9 years of age, which was stable on phenytoin. He has been diagnosed with schizophrenia. Epilepsy is present in 2 paternal half-sisters and not in a full biological brother. The mother had no mutations in the *SCN1A* gene. Father did not consent for gene testing.

A metabolic work-up included normal urine organic acids, lactate and pyruvate, and plasma amino acids. A comparative genomic hybridization (CGH) array study demonstrated an interstitial deletion at 3p26.2 (also present in normal mother). Due to unexplained seizures and encephalopathy, muscle biopsy was performed and demonstrated no histochemical or electron microscopy abnormalities. Enzymatic activity showed evidence of complex IV defect (Table 1). On gene sequencing a pathological *SCN1A* mutation, c.3734 G > A, was found that produces a missense mutation (domain 3, transmembrane region 2, external loop).[13,14]

Developmentally, he is now profoundly delayed with severe autistic behaviors. He has no expressive language and frequent self-injurious behavior treated by atypical antipsychotic drugs. On the CARS examination his score was 41, which is in the severely autistic range.

3.2. Patient 2

Patient 2 is now 6-years old. He developed febrile seizures at age 6 months and progressed to atypical absence seizures, complex partial seizures and frequent generalized tonic clonic seizures. Between ages 2–3 years, he had frequent episodes of tonic clonic status epilepticus lasting up to 1 h. An EEG at 12 months demonstrated generalized slow spike and wave complexes of 2–3 Hz. Clinically, he has a wide base gait with ataxia. There is a mild axial hypotonia present. MRI of the brain at age 1 year was normal. Until the seizures began, early development was normal. The family history was unremarkable. Initially, he was treated with phenobarbital, and subsequently with levetiracetam, topiramate, lamotrigine, carbamazepine, and ethosuximide without seizure control. At age 3 years, he was started on the ketogenic diet and had marked improvement in seizure control. Clobazam was then added resulting in further improvement, but not seizure freedom. He is currently having approximately 1 short atypical absence seizure every 2 weeks.

Metabolic work-up included normal urine organic acids, cerebral spinal fluid studies, and serum acylcarnitine profile. Plasma amino acid profile demonstrated elevation in alanine. Two venous lactate determinations were elevated (3.6, 3.0, normal <2.1 mM). Sequencing of *SCN1A* revealed a pathological mutation, c.3733 C > T, which changes arginine to a stop codon in domain 3, transmembrane region, external loop.[13,14] *POLG*, *POLG2*, and *C10orf2* sequencing were negative. Due to encephalopathy, abnormal biochemistries and seizures, a muscle biopsy was performed and found significant for a complex III defect (Table 1). There were no muscle abnormalities on histochemical or electron microscopy.

Patient 2 now has mild dysarthric speech, mild motor delay, and mild ataxia. He made significant progress in language acquisition after starting the ketogenic diet. His score on the CARS assessment was 25.5, which is not in the autistic range.

4. Discussion

Dravet syndrome is associated with *SCN1A* gene mutations in up to 90% of cases.[1,2,15,16] Mutations within *SCN1A* induce phenotypes ranging from benign febrile seizures to catastrophic epilepsy.[3] Seizure onset is in the first year of life and typically evolves into medically intractable seizures, with resultant neurocognitive regression and development of autistic behaviors.[16] Logically, one would correlate the degree of intellectual disability with seizure severity and frequency.[17] Both patients possess pathologic *SCN1A* mutations[13,14] and demonstrated the typical course of early febrile seizures progressing to medically refractory epilepsy with developmental regression. The development of patient 2 was less impacted, debatably in part, due to earlier control of status events (age 3 years versus 4 years). Patient 2 also had shorter duration of individual status events (less than 1 h versus 2.5 h). The earlier seizure control, shorter duration of status events, and younger age of improved seizure control arguably lead to improved overall developmental outcome, as evidence by his CARS score, which is not in the autistic range. Neither of our patients had prolonged atypical absence seizures, which have been associated with increased developmental involvement.[18] Age of seizure control and type of uncontrolled seizures may be a mechanism of milder cognitive impairment, but this needs to be clarified in larger studies.

Due to clinical presentation of possible mitochondrial disease, both patients were tested for mitochondrial disease by muscle biopsy. Patient 1 was tested due to progressive encephalopathy

Table 1
Electron transport chain complex activity.[a]

Patient	Complex I	Complex I/III	Complex II	Complex III	Complex IV	Citrate synthase
	29.9 ± 12.9	1.2 ± 1.1	0.8 ± 0.4	15.2 ± 6.8	148.9 ± 67.2	18.6 ± 4.7
1	28.5 (95%)	0.5 (40%)	1.3 (157%)	8.5 (55%)	30.9 (21%)	27.4 (142%)
2	17.9 (60%)	0.4 (31%)	0.7 (90%)	2.5 (16%)	70.2 (47%)	20.0 (107%)

[a] Activity: μmol/min/g wet weight.

Table 2
Clinical summary.

	Patient 1	Patient 2
Gender and age of seizure onset	Male/9 months	Male/6 months
Development prior to seizure onset	Normal	Normal
Clinic symptoms	FFT, tremor, encephalopathy	Ataxia, hypotonia
	Constipation	Encephalopathy
	Autistic behavior	Developmental delay
Seizure types	Atypical absence, drops	Atypical absence
	CPS	Tonic, CPS
Abnormal lab testing	None	Elevation Lac and Ala
Neuroimaging	Normal	Normal
ETC abnormality/CS (normalized 100%)[a]	Complex IV (15%)	Complex III (15%)
SCN1A mutation	c. 3734 G > A	c. 3733 C > T

FFT: failure to thrive; Lac: lactate; Ala: alanine; CPS: complex partial seizure; ETC: electron transport chain; CS: citrate synthase.
Both patients meet the criteria of definite mitochondrial disease.[10] Patient 1 has definite mitochondrial disease due to multiple (3 or more) system involvement (major) and <20% ETC activity (major) and Patient 2 has definite mitochondrial disease due to <20% ETC activity (major), metabolic indicators of ETC abnormality (minor) and systems compatible with ETC abnormality (minor).
[a] Activity of ETC complex is normalized to a mean control activity of marker enzyme activity of 100%, the residual activity is presented as percent of normal.

with partial seizures and was found to have complex IV dysfunction (Table 1). Patient 2 was tested for mitochondrial disease due to the presence of encephalopathy, hypotonia and biochemical abnormalities, and was found to have complex III dysfunction (Table 1). Both patients meet criteria for definite mitochondrial disease (Table 2).[10] As with our patients, the clinical presentation of mitochondrial diseases and Dravet syndrome can present similarly. Both disorders can present with normal early development followed by onset of medically refractory seizures with frequent episodes of status epilepticus, and associated developmental regression.[6,8,19] Frank autistic behaviors can be seen in patients with electron transport chain defects.[20] Our patients demonstrate the clinical similarity between the two disorders and underscore the expanding etiologies of co-morbid Dravet syndrome and mitochondrial disease.

The etiology of the co-morbidity is not entirely obvious. The etiology is clear in the patient with digenic mutations in *POLG* and *SCN1A* genes.[9] Mutations in *POLG* were not found in patient 2. Clinically, mutations in *POLG* giving rise to Alpers syndrome are not likely in patient 1 as he has been on long-term valproic acid without liver problems.[19,21,22] In addition, the lack of progressive neurocognitive decline is also suggestive that he does not have Alpers syndrome.[5] Therefore, co-morbid digenetic mutations in *POLG* and *SCN1A* do not explain our two patients. Our findings indicate that there are multiple etiologies for this co-morbidity, frank genetic mutations in genes causing known mitochondrial disease, i.e. *POLG*, and defects in the electron transport chain. The genetic components of the latter remain only partially known.

The possibility of a single mechanism for Dravet and mitochondrial diseases of our patients is not clear. As far as we understand, muscle does not express *SCN1A*. There is a report of a transactivating factor, ERRγ regulating expression of *SCN1A* and multiple mitochondrial genes in animal cardiac muscle.[23] However, it is not clear if co-regulation occurs in human tissues such as brain and skeletal muscle. Findings in our two patients would suggest that there are likely multiple mechanisms for diverse phenotypes in some patients with Dravet syndrome. Both of our patients had alterations within the *SCN1A* gene encoding the same amino acid, Arg1245. One patient had a missense mutation and the other a truncation mutation. The latter patient would logically have the more severe phenotype, however his disease was much milder. This finding would argue that there are other mechanisms influencing the *SCN1A* phenotype. Given the previous digenic mutations in *POLG* and *SCN1A* in one patient, we think it is reasonable to assume that multiple gene mutations could be altering phenotype in some patients. The finding of co-morbidity of *SCN1A* mutations and mitochondrial defects would substantiate this suggestion.

The findings of our 2 patients and the patient reported by Bolszak et al.[9] pose a seizure treatment dilemma. Valproic acid dosing is a first line therapy in Dravet patients.[24] However, valproic acid can induce liver failure if mutations in *POLG* are found.[19,25,26] Unfortunately, there are no pathogenomic clinical findings of either Dravet syndrome or Alpers syndrome to clarify when *POLG* sequencing should occur. There are some clinical clues based on EEG, biochemical, and neuroimaging findings that may help the clinician select those patients who need further workup, i.e. *POLG* sequencing or other mitochondrial diseases.[19,27–29] The use of valproic acid in other types of mitochondrial diseases related to electron transport chain deficiencies has demonstrated significant side effects and its use has been questioned.[30] Our patients suggest that co-morbid Dravet syndrome and electron transport chain deficiencies are not uncommon. Diagnosis of possible mitochondrial disease and the use of valproic likely represent a concern outside isolated digenic *POLG* and *SCN1A* mutations.

Epileptic and metabolic-induced encephalopathies are not uncommon. We report two patients who have two distinct mechanisms for progressive encephalopathies. Both have Dravet syndrome and mitochondrial disease. Our patients demonstrate the range of clinical findings that can be seen in either disease, intractable seizures, developmental delay, autism, ataxia, progressive neurocognitive involvement and add to the dilemma of phenotype:genotype for each disease. How the two disorders complement each other giving rise to disease involvement remains unknown. Much larger populations of patients with co-morbid disease are needed to tease out how each disease may complement one another in disease manifestations. The awareness of the clinician deciding when to do multiple testing is paramount to prevent medication-induced morbidity/mortality, as well as proper intervention to control seizures. Given select clinical and biochemical findings, muscle biopsy and/or sequencing of both *POLG* and *SCN1A* should be undertaken.

Disclosures

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We confirm that we have read the Journal's position on issues involved in ethical publication and affirm that this report is consistent with those guidelines.

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